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EFFECT OF INCREASING DURATION OF DIABETES MELLITUS TYPE 2 ON GLYCATED HEMOGLOBIN AND INSULIN SENSITIVITY

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ABSTRACT

Non-insulin dependent diabetes mellitus (NIDDM) is the most rapidly growing chronic metabolic disorder in the world. With advancement in the age and duration of diabetes there is a gradual tendency for the level of blood sugar to rise along with a subsequent increase in the HbA1c as well as in the fasting insulin level. Whether this is an aging process or increased frequency of diabetes is still controversial. The correlation between glucose and insulin sensitivity is consistent with the idea that the degree of chronic hyperglycemia is a cause of excessive insulin resistance in type 2 diabetes, i.e. the insulin resistance which characterizes type 2 diabetes but not non-diabetic subjects matched for age, gender, family history and duration of diabetes. The study comprised a total of 76 subjects out of which 30 were normal, non-diabetic persons and the rest 46 were diabetics with different duration of time in years, after being diagnosed diabetic. Data was analyzed after dividing the subjects into four groups - Group 1 comprised of one year old diabetics, Group 2 was made up of those, who had diabetes, for the past 2-5 years, Group 3 included patients who were diabetic since more than 5 years and Group 4 included non-diabetics as the normal control group. The results obtained indicated that the HbA1c levels showed a significant increase with the duration of diabetes as well as the insulin level showed a significant correlation after adjustment for age, sex and duration of diabetes.

KEY WORDS

Glycation, HbA1c, Insulin Resistance, Fasting Blood Glucose, Post Prandial Blood Glucose

INTRODUCTION

Diabetes mellitus is a life-long disease, which makes many people worry about the quality and longevity of their life after being diagnosed with it. The complications of diabetes are influenced not only by the duration of diabetes but also by the average level of chronic glycemia (1-2), which is measured most reliably with glycated hemoglobin assay. In normoglycemic subjects a small proportion of hemoglobin A is attached to a carbohydrate moiety thus creating what is called glycated hemoglobin (3). In conditions of sustained hyperglycemia, such as in diabetes mellitus, the proportion of hemoglobin that is glycated is increased substantially (4-5). Studies conducted by Arnetz *et al*. (6) and Kilpatrick *et al*. (7) in diabetic patients have shown a significant positive correlation between HbA1c and age as well as duration of diabetes. In contradiction to this Kabadi (8) found no significant relationship between age, duration of diabetes and fasting blood glucose (FBG), glycated hemoglobin, glycated protein or glycated albumin. According to the results of many longitudinal and cross-sectional studies it has been demonstrated that the earliest detectable abnormality in NIDDM is impairment in the body's ability to respond to insulin (9). Studies have shown that insulin sensitivity correlated inversely with fasting insulin and the insulin level increased with the duration of diabetes (10).

Though such detailed investigations have been carried out in different parts of the world to prove a correlation between the different parameters, the results were contradictory, blurring the diagnostic significance of these parameters. It is thought worthwhile to investigate the significance of such correlations in the Indian diaspora, where such a biochemical equation on the effect of these parameters for the progression of diabetic sequel has not yet been postulated. The aim of this study is to evaluate the correlation between the above detailed parameters so that they can be

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used as diagnostic or prognostic markers for the assessment of the degree of control of this lifestyle disease, to delay or prevent the multi-faceted complications before they can eventually manifest.

MATERIAL AND METHODS

The study was conducted in the Department of Biochemistry and Clinical Biochemistry of M.G.M. Medical College and OPD of M.Y. Hospital, Indore.

Clinical Material : Subjects

The clinical material for the present study comprised of a total of 76 subjects. Three groups were formed on the basis of difference in duration of diabetes. The fourth group comprised of subjects who were normal and non-diabetic, as a control group. Data on therapy of diabetes, HbA1c values, FBG and PPBG was obtained by structured questionnaires and by clinical and laboratory assessments. Insulin-sensitive subjects were defined as having an insulin-sensitivity estimate the median in non-diabetic subjects participating in the study. Using this definition 88% of all the type 2 diabetics was insulin resistant. The study subjects were established diabetics. At the time of this study the patients were not under any kind of treatment but were controlling their blood sugar level by diet and exercise.

1. **One-year old diabetics (Group 1)**

These subjects had been diagnosed with diabetes only one year before.

2. **Two-Five year old diabetics (Group 2)**

This group of patients was diabetic since the last 2-5 years.

3. **More than five-year old diabetics (Group 3)**

This group of patients was diagnosed with diabetes for more than 5 years. The maximum duration in this group was found to be of a patient with a diabetic history of 22 years.

4. **Normal subjects (Group 4)**

These individuals were screened for the presence of diabetes based on the diagnostic criteria of the American Diabetes Association (ADA) (11). They were found to be normal, healthy individuals without any prior family history of diabetes. The exclusion criteria also included hypertension, use of alcohol or cigarettes and other factors affecting blood sugar level.

Collection of material : Blood

In all the above groups 5 ml whole blood was collected in the fasting state. 0.5 ml whole blood is mixed with EDTA regent (anticoagulant) and kept for HbA1c estimation. The remaining blood is kept at room temperature for 1 hour after which the supernatant clear fluid is pipetted out into another tube. This tube is then centrifuged for 10 min. the clear serum is pipetted into a clean dry test tube and used for estimation of blood sugar and insulin. Similarly, 0.5 ml blood is collected from the subjects, 2 hours after having food for the estimation of post-prandial blood sugar, along with the urine sample.

Clinical method : estimation of HbA1c, FBG, PPBG and insulin

1. **HbA1c estimation**

For the estimation of HbA1c, 10 microliter of the whole blood + EDTA reagent, is mixed with 1ml HbA1c reagent and direct reading is taken on the auto-analyzer (Selectra E). The value recorded is in percent.

2. **FBG and PPBG estimation**

10 microliter of the clear serum is mixed with 1ml glucose reagent and incubated for 10 minutes at 37^0C. then direct reading is taken on the auto-analyzer (Selectra E). The value recorded is in milligram percent. The same process is repeated for the estimation of PPBG with the post-prandial blood sample.

3. **Insulin level estimation**

The insulin level is estimated from the clear serum separated from the fasting whole blood sample by fully Automated Radio Immuno Assay System. The value recorded is in mu IU / mL.

Statistical analysis

The statistical analysis was done by student 't' test. The values were expressed as Mean ± S.D.

OBSERVATIONS

Table 1 shows the distribution of the normal control group and the diabetic groups of people with different duration of the disease. Out of the total subjects investigated 39 (51.3%) were males and 37 (48.68%) were females. The control group included 13 (33.33%) males and 17 (45.94%) females while the number of males and females in the study group were 26 (66.66%) and 20 (54.05%) respectively. Of the total 76 cases studied, the control cases numbered to 30 (39.47%) and the total number of study subjects numbered to 46 (60.5%).

Table 2 shows the status of mean ± standard deviation of fasting insulin level, HbA1c, fasting and post-prandial blood glucose in the males and females of the study and control group.

Table 3 shows the correlation between the duration of diabetes with the various parameters, like HbA1c,

Table 1. Analysis of the 76 cases under study

Parameters	Males (n = 39)		Females (n = 37)	
	Control	Study	Control	Study
Age	55 ± 12	57.8 ± 13	59 ± 8	60.1 ± 8.2
Duration of diabetes				
0-1 Yr.	-	9	-	7
2-5 Yrs.	-	10	-	8
More than 5 years	-	7	-	5
Total	**13**	**26**	**17**	**20**

n = 76 (number of subjects / sample size)

Table 2. Insulin Level, HbA1c, FBG, PPBG values in control and study group.

Parameters	Control (n = 30)		Study (n = 46)	
Sex	Male (n=13)	Female (n=17)	Male (n=26)	Female (n=20)
Age (years)	55±12	59±8	57.8±13	60.1±8.2
Fasting insulin level (mu IU/mL)	8.2±2.1	7.4±2.0	19.2±2.8	18.4±4.2**
HbA1c (%)	2.75±0.82	2.2±0.72	7.19±2.0	6.15±1.2**
FBG (mgm%)	81.5±18.1	89±11.2	164±20.8	117±38.1**
PPBG (mgm%)	120±12.2	110±23.4	245.8±31	145.6±28.9**

**p < 0.001

Table 3. Correlation between Duration of disease, HbA1c, fasting insulin level, FBG and PPBG in control and study groups

Parameters	Control	Study Duration of Diabetes		
		0-1 yrs.	2-5 yrs.	> 5 yrs.
Fasting insulin level (mu IU/mL)	7.8 ± 3.6	10.0 ± 2.9	18.4 ± 3.2	25.95 ± 3.8*
HbA1c (%)	2.45 ± 1.2	5.9 ± 2.2	7.9 ± 3.0	12.81 ± 2.4**
FBG (mgm%)	85 ± 12.8	110 ± 10.2	180 ± 17.0	200 ± 18.2**
PPBG (mgm%)	115 ± 20.1	138 ± 12.5	210 ± 12	258 ± 10.8**

*p < 0.005; **p < 0.001

fasting insulin level, FBG and PPBG. The status of these parameters are shown in the mean ± standard deviation form.

RESULTS

The biochemical findings of this study can be expressed in the form of the following results.

1. With the increase in the duration of diabetes, the HbA1c values showed a significant increase. Males had a higher mean value of HbA1c as compared to the females.
2. Insulin level increases with the duration of diabetes though the increase is found to be within the normal limit. This means insulin resistance increases with the duration of diabetes.

3. NIDDM or the maturity onset diabetes occurs more frequently in the females at a slightly higher age as compared to the males as was shown in Table 1.

4. The fasting and post prandial blood glucose also showed a very significant increase with the duration of diabetes.

DISCUSSION

NIDDM is a chronic degenerative disease of epidemic proportions and is one of the major challenges to public health (12). India has the dubious distinction of being home to the largest number of people suffering from diabetes in any country. In theory treating diabetes should be simple, just prevent hyperglycemia from causing damage to organs and not allow hypoglycemia to cause coma as energy supply to brain fails. In practice, it does not work that way. Glucose fluctuations occur all the time and one effective way is to monitor the HbA1c, which gives the average blood glucose level of the preceding 2-3 months. In a study of 178 Libyan men it was found that the patients having poorly controlled diabetes showed a significant correlation between HbA1c and duration of diabetes (13). HbA1c will be a valuable adjunct to blood glucose determinations in epidemiological studies. In another study of 500 diabetic patients it was found that in the group of patients with HbA1c greater than 8%, there was a significant relation to the duration of diabetes (14). Various studies prove that the amount of carbohydrate attached to the HbA1c increases with increasing duration of the disease (15).

Normal levels of insulin are healthy and necessary. But too much of a good thing, in this case, insulin, can be deadly. To be healthy our body needs to produce the right amount of insulin and respond to the insulin appropriately. A confounding factor is that hyperglycemia and hyperinsulinemia in themselves can impair insulin secretion and insulin sensitivity (16, 17, 18). The body becomes more resistant to insulin with increasing duration of diabetes, so that insulin level is high or normal in the body but the available insulin is insufficient (19). As recently pointed out in a study, because of the feedback between glucose concentration (the major stimulus for insulin release) and beta-cell insulin secretion, it is virtually impossible to develop diabetes due to severity of insulin resistance found in most type 2 diabetic patients unless the capacity to secrete additional amounts of insulin to compensate for the insulin resistance is impaired (20).

The data of Table 1 shows that females suffer from diabetes at an older age as compared to males. Various other studies also proves that the disease shows a little gender preference, although diabetes becomes slightly more frequent in women with advancing age (21). Females have estrogen hormone, which is protective for developing diabetes (22), estrogen makes the body cells more receptive or sensitive to insulin. Estrogen seems to contribute to glucose homeostasis in women (23).

Poor glycemic control and age-related pathology with duration of diabetes are thought to accelerate degenerative changes in a cooperative manner (21, 24, 25, 26). The correlation analysis carried out in another study suggests that the variables like sex, age at onset of disease, duration of diabetes and age of patients influence glycemia directly and HbA1c indirectly (27).

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